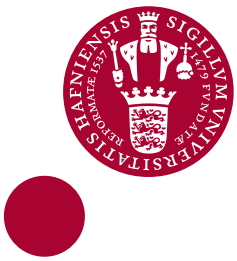

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Airway exposure to TiO_2 nanoparticles and quartz and effects on sperm counts and testosterone levels in male mice

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ABSTRACT

Several types of engineered nanoparticles (ENP) have been shown to adversely affect male reproduction in rodent studies, but the airway route of exposure has been little investigated. This precludes adequate risk assessment of ENP exposure in occupational settings. Titanium dioxide nanoparticles (TiO_2 NP) have been shown to affect total sperm count in adult male mice after intravenous and oral administration. This study aimed to investigate whether also airway exposure would affect sperm counts in male mice. Mature C57BL/6J mice were intratracheally instilled with 63 µg of rutile nanosized TiO_2, once weekly for seven weeks. Respirable α-quartz (SRM1878a) was included at a similar dose level as a positive control for pulmonary inflammation. BALF cell composition showed neutrophil granulocyte influx as indication of pulmonary inflammation in animals exposed to TiO_2 NP and α-quartz, but none of the particle exposures affected weight of testes or the epididymis, sperm counts or plasma testosterone when assessed at termination of the study.

1. Introduction

Nanotechnology may potentially increase the overall particle burden in air for workers and consumers. The introduction of particles with novel characteristics and surface reactivity may furthermore result in materials with a toxicity that differs from that of the bulk material. As engineering of nanoparticles have emerged, so has concern that these might interfere with reproductive functions [1].

Indeed, several types of engineered nanoparticles have been shown to affect male reproductive function in rodent studies (Table 1). Exposure generally reduced sperm counts, whereas changes in plasma testosterone were less consistent. The specific mechanisms underlying these observations remain to be elucidated [2]. Male reproductive function is however sensitive to inflammation and oxidative stress [2–6], both hallmarks of nanoparticle exposure [2]. Several types of nanosized particles are able to induce oxidative stress and/or inflammation, with titanium dioxide nanoparticles (TiO_2 NP) being one such example.

TiO_2 is a white pigment found in many commercial products. In bulk form, it accounts for 70% of the global production volume of pigment, but it is increasingly used in nanoform in e.g. anti-fogging and antimicrobial coatings and as absorbents of UV-light in UV-filters, sunscreens, lacquers and paints [7,8]. Airway exposure to TiO_2 nanoparticles may induce pulmonary inflammation and oxidative stress [7,8–13]. Furthermore, pulmonary deposited TiO_2 NP have been shown to translocate to secondary organs at dose levels relevant for occupational exposure [13–17]. Inhalation of TiO_2 may therefore entail systemic exposure to inflammatory mediators and translocated TiO_2 nanoparticles.

The potential for induction of lung inflammation and oxidative stress as well as translocation to male reproductive organs provide TiO_2 NP with the potential to affect male reproductive health, directly as

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Table 1
Overview over published studies investigating effects on male reproductive function of insoluble, engineered nanoparticles with stated particle size.

Particle	Species and strain (group size)	Particle size (nm)	Mode and scheme of exposure	Dose (per exposure)	Outcomes			Reference
					Sperm counts	Serum testosterone	Other	
Titanium dioxide (TiO_2,anatase)	Rat, Wistar (n = 6)	21	Intravenous injection Once weekly for 30 days Euthanized at 12-13 weeks of age	5 mg/kg 25 mg/kg 50 mg/kg	↔ SC ↔ SC ↓ SC	↔ ↓ ↓	Dose-dependent accumulation of TiO_2 in testes	[19]
Titanium dioxide (TiO_2) and Quercetin	Mouse, NMRI (n = 8)	20 – 30	Gavage Que: 42 days; TiO_2: Saline 7 days, then TiO_2 35 days; Que + TiO_2: Que 7 days, then TiO_2 35 days Euthanized at 12-14 weeks of age	TiO_2 300 mg/kg Que 7 mg/kg	↑ SC $^{\text{Que only}}$ ↓SC $^{\text{Saline+TiO}_2}$ ↓SC $^{\text{Que+TiO}_2}$	↔ Que only ↓Saline + TiO_2 ↓Que + TiO_2	Administration of Que prior to TiO_2 significantly elevated epididymal sperm count and plasma testosterone compared to exposure to only TiO_2. TiO_2 also increased abnormal sperm and testicular apoptosis and decreased sperm motility.	[18]
Cellulose nanocrystals (CNC)	Mouse, C57BL/6 J (n = 20)	L: 158 W = 54 Z: 150 (fibrous)	Pharyngeal aspiration Twice weekly for 3 weeks Euthanized at 20 weeks of age	40 μg	↓ SC	↑ (in serum and testicles)	Histology: edema, dystrophic seminiferous tubules and arrested spermatogenesis Inflammatory markers: Serum: ↑: IL-1β, IL-2, IL-2p40, KC, MCP-1, TNF-α Testicles: ↑: IL-1α, IL-1β, IL-2, IL-2p70, TNF-α, KC, MCP-1, RANTES	[39]
Carbon black	Mouse, ICR (n = 15-16)	14, 56 or 95	Intratracheal instillation Once weekly for 10 weeks Euthanized at 16 weeks of age	100 μg/exposure	↓ $DSP^{14\ nm}$ ↓ $DSP^{56\ nm}$ ↓ $DSP^{95\ nm}$	↑ 14 nm ↑ 56 nm ↔ 95 nm		[40]
Ferric oxide (FeO_3)	Mouse, albino (n = 6)	< 50	Intraperitoneal injection Once weekly for 5 weeks Euthanized at 13 weeks of age	25 mg/kg 50 mg/kg	↓ normal sperm	↑	Histology: dose-dependent accumulation of FeO_3 in testes	[41]
Silica (SiO_2)	Mouse, C57BL/6 J (n = 10)	57.6	Tracheal perfusion Every 3rd day for 45 days (15 doses) Euthanized at 13 and 17 weeks of age	2 mg Si-NP/kg Euthanized: 45 and 75 days after first dose	↓ SCC $^{45\ days}$ ↔SCC $^{75\ days}$	—		[42]
Silica (SiO_2)	Mouse, ICR (n = 8)	64 Z: 107.5	Intravenous injection Every 5th day over 15 days Euthanized at 10, 13 and 17 weeks of age	20 mg/kg Euthanized days 15, 35 and 60 after first dose	↓ SCC $^{15\ days}$ ↔ SCC $^{35,\ 60\ days}$	↔ $^{(15,\ 35,\ 60\ days}$		[43]
Silica (SiO_2)	Mouse, BALB/c (n = 5)	70 300	Intravenous injection 70 and 300 nm: two doses over two consecutive days. Euthanized after 24 hours. 70 nm only: four intravenous injections every other day. Euthanized at 48 hours or 1 week after last administration	0.4 mg/kg$^{70+300nm}$ 0.4 + 0.8 mg/kg$^{70\ nm}$	—	—	Presence of Silica-NP (70 nm) in testes (SC and spermatocytes), 24 hours after injection. No presence of 300 nm Silica in testes 24 hours after injection No histologic changes in mice exposed to 0.4 or 0.8 mg/kg 70 nm Silica neither 48 hours nor 1 week after first injection.	[44]

↓ = significant decrease, ↑ = significant increase, ↔ = no significant effect compared to control animals, ns = not stated, SC = sperm count, SCC = sperm concentration, DSP = Daily sperm production, L: length, W: width, Z = mean hydrodynamic diameter, IL = Interleukin, TNF-α = Tumor-Necrosis Factor α, Que = Quercetin.

well as indirectly. When mice were orally exposed to TiO_2 NP (300 mg/kg for 35 days), the level of oxidative stress increased in the testicles, histological changes appeared in testicular tissue, and sperm quality and testicular and serum testosterone decreased. Preemptive oral administration of the antioxidant quercetin however attenuated these effects [18], indicating that oxidative stress may be a driving force in compromising male reproductive parameters.

Another study assessed male reproductive toxicity of 21 nm TiO_2 NP after intravenous exposure of adult rats to 0, 5, 25 or 50 mg/kg once weekly for 30 days. Total sperm counts were reduced at 50 mg/kg, and serum testosterone and relative testicular weight at 25 and 50 mg/kg. Most interestingly, TiO_2 NP accumulated dose-dependently in the seminiferous tubules [19].

Concern has been raised with regard to occupational exposure and safety for workers, especially during the production phase where TiO_2 exposure mainly occurs via the airways [20]. The airway route of exposure has however been applied only in few studies of male reproductive function, precluding adequate risk assessment of NP exposure in occupational settings.

Based on the characteristics of TiO_2 particles as well as the findings in studies using the oral and injection routes of exposure, we hypothesized that also airway exposure to TiO_2 NP would affect male sperm count and possibly plasma testosterone levels. We exposed mature mice to TiO_2 NP by intratracheal instillation at occupationally relevant exposure levels during a full spermatogenic cycle, followed by assessment of reproductive organ weights, sperm count and plasma testosterone. To investigate the effects of particle-induced inflammation, a second group of animals were exposed to a larger respirable α-quartz particle, which is a very potent inducer of oxidative stress and pulmonary inflammation [9,21] and likely too large for substantial translocation across the blood-testis barrier [22].

2. Materials and methods

2.1. Animals

Forty seven male C57BL/6 J BomTac mice (Taconic Europe, Ejby, Denmark) were acclimatized for one week before the commencement of experimental procedures at 9 weeks of age. The animals were housed five to a cage as previously described [11,23], in polypropylene cages with bedding and enrichment nesting material (Enviro Dri, Lillico Biotechnology, UK), mouse house and small aspen blocks (Tapvei, Estonia)]. Environmental conditions were controlled (light 06:00–18:00) and food (Altromin 1324, Brogaarden, Denmark) and tap water were provided ad libitum. All experimental procedures followed the handling guidelines established by the Danish government and permit was obtained from the Experimental Animal Expectorate (no. 2012/15-2934-00223).

2.2. Particle characteristics, exposure preparation and characterization

The TiO_2 NP used in this study, UV-Titan (L181, Kemira, Finland), has been characterized and described previously [11]. It consists of 70% rutile TiO_2 and 30% w/w surface modifications by silicon (5.6%), aluminum (2.4%), zirconium (8.6%) and polyalcohols, has a nominal particle size of 17 nm and a specific surface area of 107.7 m^2/g [11]. The particle was chosen to act as a reference particle for particles used in paint [7]. SRM1878a consists of 100% SiO_2, (93.7% α-quartz and 6.3% amorphous silica) [24] and was chosen as a positive control for pulmonary inflammation.

Particle suspensions were freshly prepared on each day of instillation. Particles were suspended in Nanopure Diamond UV water to 2.56 mg/mL and sonicated for 16 min as described in [25]. The stock dispersion was then further diluted with Nanopure water to 1.28 mg/mL and sonicated for another 2 min. All mice were exposed within 30 min after the final sonication using Nanopure water as a vehicle and in sham instillation of control animals. The hydrodynamic particle size distribution in Nanopure water was measured by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS equipped with 633 nm He-Ne Laser (Malvern Inc., UK) as described previously [34].

2.3. Animal exposure

The mice were semi randomized into three groups with similar weight distributions (n = 15–16): vehicle control (Nanopure water), UV-Titan, and SRM1878a. Power analysis showed that a group size of 15 animals per group would allow for detection of a 20% difference in sperm count per gram testicular tissue (SC/g) between a control and an exposed group with a probability of 80%, based on a significance level of 0.05 and standard deviations as reported in Kyjovska et al. (2013) [26,27].

Animals in the particle groups were intratracheally instilled with 63 μg of particle once a week for seven consecutive weeks (total dose 441 μg), under isoflurane anesthesia (3–4%). The deposited volume was 50 μl, which was followed by 150 μl of air [28]. Water was chosen as vehicle to avoid an eosinophilic response to repeated instillation of dispersion agents [29].

The weekly dose of UV-Titan was chosen to represent the estimated lung deposition of titanium at the Danish occupational exposure limit of 6 mg Ti/m^3 air [30]. For comparison, a similar exposure level was applied for quartz, albeit the dose level was relatively higher compared to the Danish occupational exposure limit which is 0.1 mg respirable quartz/m^3 air) [30].

2.4. Euthanasia and organ collection

One week after the final instillation, i.e., 8 weeks after the initial instillation, the animals were exsanguinated under deep anesthesia with a cocktail of ZRF (Zoletil® 250 mg/mL, Rompun® 20 mg/mL and Fentanyl® 50 mg/mL in sterile isotone saline) at a dose of 0.01 mL/g body weight. Lungs, heart, liver, testicles, epididymides and brain were harvested, weighed, snap frozen in liquid nitrogen and stored at −80 °C.

2.5. Bronchoalveolar lavage

Bronchoalveolar lavage fluid (BALF) was collected by cannulating the trachea with a 22 gauge BD Insythe catheter and flushing the airways twice with 0.8 mL of 0.9% saline in a 1 mL syringe. Fresh saline media was used for each round. BALF was stored on ice until centrifuged at 400 *g* at 4 °C for 10 min and the pellet was re-suspended in 100 μL of Ham's F-12 nutrient Mix cell culture medium (Thermo Fisher Scientific, Denmark). Total cell counts were measured by NucleoCounter (NucleoCounter NC-200, Chemometec, Denmark). 50 μL of the BALF cell suspension were pipetted onto glass slides and spun at 1000 rpm for 4 min in a cytospin centrifuge. Differential cell counts were performed under a bright field microscope by counting 200 cells fixed and stained as described previously [31]. All slides were randomized and blinded before scoring by the same person.

2.6. Sperm counts

One week after the last exposure, the study was terminated and sperm was counted as assessed as described by Kyjovska et al., (2013) [27], in whole testicle and per gram testicular tissue. For the analysis, the left testicle was decapsulated and the parenchyma homogenized in 4.0 mL homogenization buffer (0.9% NaCl and 0.05% Triton X-100 (Sigma, Germany)). Spermatids were stained with 0.04% trypan blue (GURR, Hopkin and Williams, UK) and the stage 14–16 spermatids (as it is these stages that are resistant to homogenization) were counted under microscope at 400x magnification using a haemocytometer (Bürker chamber, 0.0025 mm^2, depth 0.1 mm). Each sample was

counted in replicates and averaged before calculating the total number of spermatids per testicle. The spermatid number per g testicular parenchyma (SC/g) was calculated by dividing the total number of spermatids with testicular weight. [27].

2.7. Testosterone analysis

Blood was collected by cardiac puncture of anesthetized animals, stabilized with ethylenediaminetetraacetic acid (EDTA, 36 µL) and centrifuged at 2500 *g* for 10 min. The resulting plasma was stored in aliquots at – 80 °C until analysis. Plasma testosterone was quantified in duplicates in 1:2 dilution with Phosphate Buffered Saline (PBS, 0.01 M, Sigma Aldrich) using a competitive Enzyme-Linked Immunosorbent Assay (RTC001R, Biovendor, Czech Republic). Samples were analyzed following the manufacturer's protocols, with a standard curve from 0.1 to 25 ng/mL. Samples that fell above the standard curve were diluted 1:4 in PBS and re-analyzed. Absorbance was read at 450 nm with a microplate reader (RT-6500, Rayto, Shenzhen, China). The assay had been validated prior to application in the present study (CV: 4.8–7.8%) [32]. Results were interpolated using 4 PL regression with GraphPad Prism 7.0 (GraphPad Software, La Jolla, California, USA).

2.8. Behavior in the open field

The animals were tested in the open field test, to screen for effects on general well-being in supplement to daily routine observation of the animals, and, potentially, on central nervous system function. The day before termination of the study, mice were transferred to the experimental room 1 h before the first test session, during the light period, with the observer blinded to animals' exposure status. One animal was placed centrally in the circular arena (Ø: 1 m), and activity was assessed for three minutes. The location of the animal was traced and registered by Noldus Ethovision XT (v. 5, Noldus Information Technology, Wageningen, Netherlands) that also calculated total ambulation, split into time-bins of 1 min to test for habituation, duration in the central and peripheral zones of the field, and the number of crossings between these zones.

2.9. Statistics

Prior to the statistical analysis, data was investigated for normal distribution. Normally or lognormally distributed data was analyzed by one-way ANOVA, followed by Dunnet's Multiple Comparisons test when appropriate. Non-normally distributed data (neutrophil, lymphocyte and eosinophil counts) were analyzed by the Kruskal-Wallis test, followed by Dunn's multiple comparisons test. Open field data was analyzed in SYSTAT Software Package 9, all other analyses with Graphpad Prism 7.0 (GraphPad Software).

3. Results

3.1. Exposure characterization (DLS)

Average hydrodynamic diameter (Z-average) and polydispersity indexes for UV-Titan and SRM1878a in the vehicle solution are shown in Table 2. The hydrodynamic number size distributions show a large peak at 91 nm and a smaller peak at 33 nm for UV-Titan, and a single peak at 255 nm for SRM1878a (Fig. 1).

Table 2
Nominal size, hydrodynamic diameter and polydispersity indexes of UV-Titan and SRM1878a in 1.28 mg/mL Nanopure water.

Particle	Nominal size	Z-average	PDI	Reference
UV-Titan	17 nm	218 nm	0.24	[11]
SRM1878a	1.6 µm	359 nm	0.26	[24]

Z-average is the average of 7 measurements by DLS, PDI = Polydispersity index

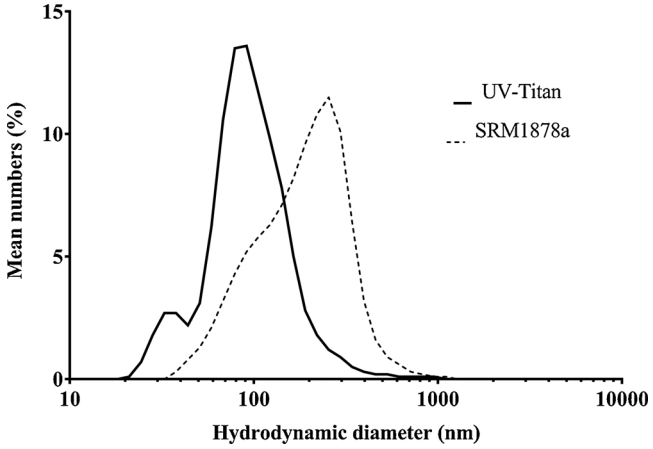

Fig. 1. Mean number distribution of 1.28 mg/mL UV-Titan and SRM1878a in Nanopure water (n = 7).

3.2. Body and organ weights

Absolute and relative weights of lungs of mice exposed to SRM1878a were significantly elevated when compared to the vehicle control group. No other differences were observed (Table 3).

3.3. Pulmonary inflammation

BALF cell composition and neutrophil granulocyte influx were used as indices of lung inflammation (Fig. 2). One week after the final instillation, pulmonary inflammation was evident, based on the significantly elevated counts of total cells, macrophages, neutrophil granulocytes and lymphocytes in animals exposed to UV-Titan and SRM1878a compared to control animals ($P < 0.001$). Furthermore, SRM1878a induced a significantly larger neutrophil influx than UV-Titan ($P < 0.01$).

3.4. Sperm counts

Sperm counts in whole testicle as well as per gram testicular tissue were similar in vehicle control and exposed animals (Table 4).

3.5. Testosterone

Plasma testosterone levels was 6.56 ± 2.9, 5.48 ± 2.6, and 11.2 ± 2.9 ng/mL for vehicle controls, UV-Titan and SRM1878a, respectively (mean ± SEM). The higher level in the SRM1878a group owes to a slight overweight of animals in the higher range of values (Fig. 3), but no statistically significant difference was observed in pairwise comparison of particle exposed groups compared to controls (overall analysis, $P = 0.074$; SRM1878a compared to controls, $P = 0.170$).

3.6. Behavior in the open field

No differences were observed in the open field test (data not shown).

4. Discussion

The present study investigated the effects of airway exposure to TiO_2 NP and quartz particles on male reproductive parameters. The

Table 3
Body weight and gain, absolute and relative organ weights at termination of study.

		Vehicle Control	UV-Titan	SRM1878a
Body weight	(g)	31.7 ± 0.4	31.8 ± 0.7	31.4 ± 0.5
Body weight gain	(g)	5.50 ± 0.34	5.29 ± 0.34	6.07 ± 0.34
Heart	(mg)	165.4 ± 3.6	164.1 ± 5.3	170.1 ± 5.3
	(%)	0.52 ± 0.01	0.52 ± 0.01	0.54 ± 0.02
Lung	(mg)	343.7 ± 11.5	339.9 ± 6.8	382.8 ± 11.1 *
	(%)	1.08 ± 0.03	1.08 ± 0.03	1.22 ± 0.04 **
Liver	(mg)	1482 ± 57	1413 ± 42	1398 ± 41
	(%)	4.66 ± 0.13	4.44 ± 0.08	4.45 ± 0.10
Brain	(mg)	346.9 ± 12.4	329.1 ± 5.4	334.4 ± 8.8
	(%)	1.10 ± 0.04	1.04 ± 0.02	1.07 ± 0.03
Testes	(mg)	235.7 ± 5.0	246.5 ± 3.7	238.4 ± 3.2
	(%)	0.74 ± 0.02	0.78 ± 0.01	0.76 ± 0.01
Epididymides	(mg)	118.6 ± 7.3	125.8 ± 11.8	131.3 ± 8.1
	(%)	0.38 ± 0.03	0.39 ± 0.03	0.42 ± 0.03

Mean ± SEM (n = 15–17). *P < 0.05, **P < 0.01 compared to the vehicle control. Weights of paired organs (lungs, testes and epididymides) are presented as the mean of the paired organs.

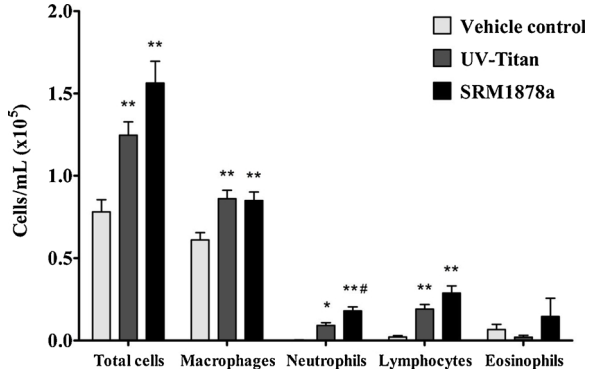

Fig. 2. Pulmonary inflammation presented as total cells, macrophages, neutrophils, lymphocytes and eosinophils in BALF 7 days after the final instillation. Mean ± SEM (n = 15–16). *P < 0.01, **P < 0.001 compared to vehicle control. #P < 0.01 compared to UV-Titan.

Table 4
Sperm count in whole testis and per gram testicular tissue in male C57BL/6 J mice one week after the final instillation of particles.

	Vehicle control	UV-Titan	SRM1878a
Whole testicle (x10^6)	17.4 ± 0.5	18.4 ± 1.0	17.6 ± 0.6
SC/g (x10^7)	16.1 ± 0.45	16.6 ± 0.7	16.5 ± 0.6

Mean ± SEM (n = 15 – 16). SC/mg = sperm counts per g testicular tissue.

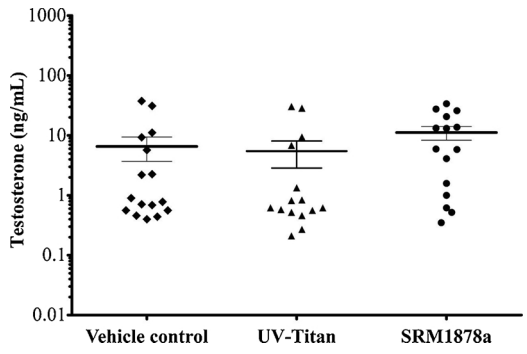

Fig. 3. Plasma testosterone levels in male C57BL/6 J mice one week after the final instillation of particles. Mean ± SEM (n = 15–16).

strengths of the study include an occupationally relevant dose level, exposure during a full spermatogenic cycle, the use of validated methods for analysis of plasma testosterone and daily sperm production [27,32], and sufficient statistical power.

We had hypothesized that airway exposure to TiO_2 NP would affect male reproductive parameters. Both TiO_2 NP and α-quartz induced pulmonary inflammation, α-quartz more so than TiO_2 NP. The value of plasma testosterone was numerically, but not statistically significantly, higher in mice exposed to SRM1878a compared to vehicle and TiO_2 exposed mice. Sperm counts remained unaltered in both the TiO_2 and SRM1878a exposed groups as did absolute and relative weights of the testes and epididymides, i.e. neither particle interfered with the assessed indicators of male reproduction. Overall, the data indicate that sperm count is not susceptible to nanomaterial exposure via the airways at the applied dose levels. It should however be kept in mind that the assessed outcomes provide only a partial characterization of the male reproductive parameters, as important features such as testicular and epididymal histology were not assessed. We did also not evaluate whether the males were able to fertilize females.

We had based our hypothesis on the findings by Meena et al (2014) [19], exposing adult male Wistar rats intravenously to 21 nm anatase TiO_2 NP once weekly for four weeks and reporting reduced sperm counts at an accumulated dose of 200 mg/kg. The accumulated dose was 17 mg/kg in our study, i.e., much lower than the effective dose in Meena et al. (2014) [19]. As only a small fraction of the instilled particles would be expected to translocate from the lungs to the bloodstream [17,33], the direct testicular exposure would be much lower in the current study.

As a secondary hypothesis, we proposed that particle induced lung inflammation by itself would affect male reproductive parameters. This was investigated by exposing a second group of males to SRM1878a, a known and documented potent inducer of oxidative stress and pulmonary inflammation [9,21], as its larger nominal size would reduce translocation across the blood-testes barrier compared to TiO_2 and therefore the potential for direct effects on the testicles. Exposure characterization by DLS showed the average hydrodynamic diameter of SRM1878a to be smaller than expected from its nominal size, although the mean diameter of 255 nm would still preclude substantial translocation across the blood-testes barrier. Although pulmonary inflammation was evident, and higher than for TiO_2, also lung exposure to SRM1878a left the assessed male reproductive parameters unaffected.

Overall, our findings agree well with a report of pulmonary inflammation without effects on sperm counts and plasma testosterone following instillation of adult male mice to four different carbonaceous NPs, once weekly for seven weeks, albeit at a slightly higher exposure level [34].

Inflammation induced by administration of lipopolysaccharide is known

to depress male reproductive function in animal studies [3,35], and male reproductive function is generally considered sensitive to inflammation [4]. The reason why inflammation does not affect sperm parameters in our study is unexplained. Khorsandi et al. (2017) found the antioxidant quercetin to ameliorate changes in male reproductive parameters otherwise induced by oral exposure to TiO_2 NP [18]. This indicates that oxidative stress, arising as a consequence of testicular inflammation, could be a driving force for augmented male reproductive function. Proinflammatory cytokines have furthermore been hypothesized to increase the permeability of the blood-testes barrier, leaving the testicles more prone to translocation of particles present in the blood across the blood-testes barrier [36–38]. Interestingly, androgens such as testosterone, have been hypothesized to help restore the integrity of the blood-testes barrier in the presence of proinflammatory cytokines (reviewed in [36–38]). Increased plasma testosterone may therefore act as a protective factor in the presence of inflammation. Several previous studies indeed report increased levels of plasma testosterone following exposure of male mice to nanoparticles, in the presence of reduced male reproduction [39–41], in some with confirmed increases in the levels of proinflammatory cytokines and markers of oxidative stress [39,41]. In our study, plasma testosterone was numerically, but statistically insignificantly, increased in mice exposed to SRM1878a. This group also presented with the strongest pulmonary inflammatory response. The relationship between testosterone and inflammation has however still to be elucidated.

5. Conclusion

The present study applied validated methods for analysis of sperm counts and plasma testosterone, was adequately powered and covered a full spermatogenic cycle. Despite several proposed mechanisms on the male reproductive toxicity of engineered NP, our results do not indicate that airway exposure to rutile TiO_2 or quartz at occupationally relevant dose levels affect sperm counts and plasma testosterone, at least not in young adult males.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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